Applicants' Response

Applicants' response to the office action of 12/05/2005 includes two sections: for each rejection: (1) a restatement of the Examiner's position which is found in 10 point italic font, and (2) applicant's reply in 12 point normal font.

Response to Amendment

The amendment to the claims filed on 04/05/2006 was found not to comply with the requirements of 37 CFR 1 .121(c) because the Applicants indicate the status of "original" for the amended claim 43 wherein it was required to indicate the correct "currently amended" status for claim 43.

Applicants have changed the status of claim 43 to show it as being currently amended. Applicants' apologize for not catching this error in their last submission.

Response to Arguments

Rejections under 35 USC § 112, first paragraph

Claims 1-4, 7-26, 39, 43, and 44 remain rejected and new claims 45-47 are rejected under 35 USC § 112 first paragraph as not being enabled for (i) a therapeutic delivery system comprising an implanted electrical pulse generator operably coupled with genetically engineered cells that have been transplanted into a mammalian tissue, wherein said genetically engineered cells further comprise a target gene that has been operably coupled in vitro to a heterologous electrically responsive promoter capable of enhancing transcription of said target gene (claims 1-4, 7-26, and 43) and (ii) a method of treating a patient comprising providing the patient with an implantable electrical pulse generator operably coupled with genetically engineered cells that have been transplanted into a patient tissue, wherein said genetically engineered cells further comprise a target gene operably coupled in vitro to a heterologous electrically responsive promoter capable of enhancing transcription of said target gene (claims 39, and 45-48), for the reasons of record set forth in the prior Office Action.

Applicant's arguments filed 04/05/2006 have been fully considered but they are not persuasive. Applicants had traversed the previous rejection on the grounds that they submitted a series of amendments to the independent claims 1 and 39. More specifically, the claims were amended to recite that (i) the genetically engineered cells comprise the target gene operably coupled in vitro to an electrically responsive promoter, (ii) the electrical pulse generator is implanted, (iii) the genetically engineered cells are implanted in a mammalian tissue, (iv) the system is capable of enhancing transcription, and (v) the electrically responsive promoter is a heterologous promoter. Applicants had argued that the Examiner stated that the application was enabled under these conditions and, therefore, the present rejection should be withdrawn.

Contrary to Applicants assertion, the Examiner points out those amendments to claim 1 and 39 are not sufficient to overcome the enablement rejection of the instant claims for several reasons.

First, the Examiner clarifies that the instant claims are only enabled for (i) electrically stimulated induction of gene expression in vitro using an electrical pulse generator operably coupled

with cultured genetically engineered cells comprising a target gene operably coupled to an electrically responsive promoter (i.e. in vitro induction of gene expression by electrical stimulation using an electrical pulse generator operably coupled with cultured genetically engineered cells comprising a target gene operably coupled to an electrically responsive promoter) and (ii) delivering to a subject an electrical pulse generator operably coupled to genetically engineered cells, wherein genetically engineered cells are transplanted into the subject and not for the treatment of a patient. Although they were amended, claims 1-4, 7-26, and 43 were still drawn to a therapeutic delivery system, and therefore the amendments to the claims are not enough to overcome the instant rejection. Such language directed to a therapeutic delivery system was considered to directly embrace administering to animals a therapeutic agent in an amount sufficient such that the treatment of an animal having a condition associated with the therapeutic agent is achieved. Accordingly, preamble language directed to "therapeutic delivery system" is considered to require support as outlined in 35 USC § 112 first paragraph such that therapeutic benefit is considered to be enabled for one seeking to make and use such a delivery system . Similarly, claims 39, and 43 and new claims 45-47 were found still drawn to a method of treating a patient by using the therapeutic delivery system. Claims 1-4, 7-26, 39, 40, and 43 were not considered enabled for the treatment of a patient because issues with the breadth of the claims (the range of diseases to be treated, the range of therapeutic agents, and the range of genetically modified cells), with the unpredictability of the outcome of the treatment, with prolonged and/or controlled expression of the therapeutic gene in a sufficient amount to result in a therapeutic effect, and with the lack of guidance and working examples still remain (see the prior Office Action). The same applies to the new claims 45-47, which are dependent on claim 39.

Second, the Examiner points out that amending the claims to recite that the genetically engineered cells comprise "a target gene that has been operably coupled in vitro to a heterologous electrically responsive promoter" means only that the construct was recombinantly made in vitro. Therefore, the genetically engineered cells can be obtained either in vitro by transfecting them with the recombinant construct before transplantation, or in vivo by transfecting the pre-transplanted cells via the delivery of the construct to the mammal. As a consequence, issues such as unpredictability of the efficacy of delivering the construct to the targeted cells (i.e., delivery systems, specific delivery to the desired cells, and target accessibility) still exist (see prior Office Action).

Given the diverse and unpredictable outcome of using the disclosed delivery system to treat diseases, the Examiner finds that the specification does not appear to provide sufficient guidance and/or working examples that specifically address the use of this delivery system as being effective in treating various diseases in animals to enable one of ordinary skill in the art to use such delivery system without undue experimentation.

In conclusion, the presently claimed invention only provides enough of a disclosure to allow for an Artisan to: (i) electrically stimulate, in vitro (i.e., in tissue culture), induction of gene expression using an electrical pulse generator operably coupled with cultured genetically engineered cells comprising a target gene operably coupled to an electrically responsive promoter and (ii) delivering to a subject an electrical pulse generator operably coupled to genetically engineered cells, wherein genetically engineered cells are obtained in vitro and then transplanted into the subject.

In view of the Examiner's remarks, Applicants have removed reference in the pending claims to a therapeutic system or patient and have focused the claims to a delivery system. Examiner has previously indicated that the claims directed to such a delivery system would be considered enabled. Applicants request reconsideration and removal of the present rejection of the claims under 35 USC § 112, first paragraph.

New Rejections

Specification

Claim 9 was objected to under 37 CFR 1 .75(c), as being of improper dependent form for failing to further limit the subject matter of a previous claim . Applicant is required to cancel the claim(s), or amend the claim(s) to place the claim(s) in proper dependent form, or rewrite the claim(s) in independent form. Claim 9 recites "a therapeutic delivery system of claim 1 wherein the electrical pulse generator is external". However claim 1 is drawn to an implanted electrical pulse generator and does not disclose an external electrical pulse generator.

Applicants have cancelled claim 9, thus rendering the present rejection moot.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Rejection under 35 USC 103(a) as being unpatentable over Lee et al. in view of both Kanno et al. and Pahwa et al.

Claims 1-4, 7-10, 13, 14, 23-25, 39, 40, and 43-48 were rejected under 35 U.S.C. 103(a) as being unpatentable over Lee et al. (Circulation, Aug, 22, 2000, 102: 898-901), in view of both Kanno et al. (Circulation, 1999, 99:1682-1687) and Pahwa et al. (Neurology, 1997, 49:249-253). Lee et al. teach implantation of primary murine myoblasts expressing the murine VEGF gene from a retroviral promoter into the ventricular wall of immunodefficient mice (Abstract, p. 899, column 1, Materials and Methods). Lee et al. was cited for teaching that uncontrolled VEGF expression from the implanted VEGF-expressing skeletal muscle myoblasts results in the formation of hemangiomas and therefore, a regulated VEGF expression are needed for a successful therapy (p. p. 898, column 2 bridging p. 899, p. 900, column 2, last paragraph). Lee et al. was indicated by the Examiner not to specifically teach an electrically responsive promoter or induction of VEGF by electrical stimulation. Kanno et al. was cited for teaching induction of VEGF in electrical pulse stimulated murine myoblast cell line C2C12, i.e., the VEGF gene comprises an electrically responsive promoter (p. 1682, column 2, Methods, and p .2684, column 2, second and third paragraphs). The pulses were considered not to damage the cells and thus, were considered to be sub-threshold- or threshold-applied pulses. Kanno et al. was cited for teaching that gene therapy using VEGF is relevant for therapeutic angiogenesis and that localized electrical stimulation could force cells in the ischemic area to synthesize an adequate amount of VEGF to salvage the ischemic area (Abstract, p. 2686, column 2, last paragraph). Therefore, the Examiner concludes it would have been obvious to one of skill in the art, at the time the invention was made, to modify the procedure of Lee et al. and increase VEGF expression by generating an electrical pulse, as taught by Kanno et al., with a reasonable expectation of success. The Examiner cites that the motivation to do so is provided by Kanno et al. who teaches that gene therapy as relevant for therapeutic angiogenesis and the importance of localized, controlled expression of VEGF induced by electrical pulse stimulation that can promote the activity of the

promoter which would activate local VEGF production, salvaging the ischemic area (Abstract, and p. 2686, column 2, last paragraph) and by Lee et al. who teach potential toxicity of unregulated myoblasts-mediated VEGF expression (p. 900, column 2, last paragraph). However, the Examiner points out that neither Lee et al. nor Kanno et al. explicitly teach the limitation of using an implanted pulse generator operably coupled with the VEGF-expressing myoblasts as to provide an electrical pulse for gene expression in these cells . However, at the time the invention was made, Pahwa et al. did teach that electrical pulse generators could be implanted to stimulate a targeted tissue and that they can be externally controlled by computers (Abstract, p . 250, column 2, p. 251, column 1) . Thus, the Examiner concludes it would have been obvious to one of ordinary skill in the art to employ such devices for providing a desired amount of pulses to the targeted tissue (i.e., the genetically engineered implanted myoblasts) as taught by the primary reference, e.g., Lee et al. or Kanno et al. and that one of ordinary skill in the art would have been motivated to employ a pulse making device such as an implanted or external pace maker or pulse generator because such devices are well known in the art and the use of the device would provide the sources of pulses as required for providing a stimulation of gene expression, which electrical pulse stimulation of a promoter is crucial for modulation of gene expression as taught by the primary reference. One of ordinary skill in the art would have a reasonable expectation of success in making and use such as the combined composition because medical devices such as implantable pulse generators or pace makers are proven to provide any amount of pulses as desired in stimulating gene expression. With respect to the limitation of a heterologous electrically responsive promoter, absent evidence of unexpected results, if the general conditions of a given method are disclosed in the prior art, it would have been obvious to the ordinary skilled artisan to vary the parameters in a given method (i.e., replace the VEGF electrically responsive promoter with a heterologous one) with the aim of optimizing the results. Again, absent evidence to the contrary, it is generally not inventive to substitute equivalents known for the same purpose, such substitutions can be done by routine experimentation. The following is a citation from MPEP:

2144 .06 Art Recognized Equivalence for the Same Purpose

> SUBSTITUTING EQUIVALENTS KNOWN FOR THE SAME PURPOSE
> *In order* to rely on equivalence as a rationale supporting an obviousness' rejection, the equivalents must be recognized in the prior art, and cannot be based on applicant's disclosure or the mere fact that the components at issue are functional or mechanical equivalents. *In re Ruf,* 256 F .2d 590, 118 USPQ 340 (CCPA 1958). (The mere fact that components are claimed as members of a Markush group cannot be relied upon to establish the equivalence of these components. Moreover, an applicant's expressed recognition of an art-recognized or obvious equivalent may be used to refute an argument that such equivalency does not exist); *In re* Scotr, 323 F .2d 1016, 139 USPQ 297 (CCPA 1963) (Claims were drawn to a hollow fiberglass shaft differed from archery and a process in the production thereof where the shaft differed from the prior art in the use of a paper tube as the core of the shaft as compared with the light wood or hardened foamed resin core of the prior art . The Board round the claimed invention would have been obvious, reasoning that the prior art foam core is the functional and mechanical equivalent of the claimed paper core . The court reversed, holding that components *which* are functionally or mechanically equivalent is not necessarily obvious in view of one another, and in this case; the use of a light wood or hardened foam resin core does not fairly suggest the use of a paper core.); Seim v. *Hauchi,* 209 USPQ 754 (Bd. of Pat Inten 1950) (The mere fact that phthalocyanine and selenium function as equivalent *photoconductors* in the claimed environment was not sufficient to establish that one would have been obvious over the other. However, there was evidence that both phthalocyanine and selenium were known photoconductors in the art of elect photograpby . "This, in our view, presents strong evidence of obviousness in substituting one for the other in an electrophotographic environment as a photoconductor." 209 USPQ at 759 .). An express suggestion to substitute one equivalent component or process for another is not necessary to render such substitution obvious . *In re* Fate. 675 F .2d 297, 213 USPQ 532 (CCPA 1982).

Thus, the claimed invention was prima facie obvious at the time the invention was made.

Applicants respectively traverse that the claimed invention is unpatentable over Lee et al. in view of Kanno et al. and Pahwa et al. because 1) neither of these

references singularly or in combination teach the claimed invention or 2) that there is sufficient motivation to combine these references, or 3) that the stimulation device of Pahwa et al. is an art recognized equivalent of Kanno et al.

As the Examiner points out, Lee teaches delivery of a VEGF gene through a retroviral promoter into the ventricular wall of immunodefficient mice. However, no where does Lee teach that the retroviral promoter is an electrically responsive promoter. In order to overcome the deficiencies of Lee, the Examiner has cited the reference by Kanno et al. Kanno et al. was cited for teaching the induction of the VEGF gene in electrical pulse stimulated C2C12 cells as an electrically responsive promoter system.

One skilled in the art reading Kanno et al. would find that Kanno et al. only teaches induction of expression of the endogenous VEGF gene using electrical stimulation in non-genetically engineered cells. It was not shown in Kanno et al. to be effective in engineered cells, nor was it shown to be applicable in heterologous promoter systems. Both these limitations are contained in the claims.

In addition, contrary to the Examiner's assertion, after reading Kanno et al. there would be no motivation for transfecting the VEGF gene into the tissue to enhance expression of this gene by electrical stimulation, wherein the gene is already present in the tissue and responsive to electrical stimulation. Because the gene is present and shown to be responsive to electrical stimulation there is no motivation to make a heterologous promoter system. Electrical stimulation of the endogenous gene would have achieved the desired result without creating the required genetically engineered cells. Neither Lee et al. or Kanno teach heterologous promoter constructs using an electrically responsive promoter element.

Another deficiency of Lee et al. and Kanno et al. is that neither teaches the use of an implantable pulse generator to stimulate the implanted cells. The Examiner recognizes this deficiency of the Lee et al. and Kanno et al. In order to overcome this deficiency, the Examiner cites Pahwa for teaching the electrical pulse

generator and argues this is an art recognized equivalent to the device cited in Kanno.

Applicants disagree that under the cited cases provided in MPEP 2144.06 that these two systems are art recognized equivalents. First, there is no art recognized equivalent between the devices taught in Kanno compared to those cited and Pahwa. One skilled in the art would not find that this is a matter that one device can be substituted for the other. This is not the same situation as found in the Smith v. Hayashi case where it was determined that phthalocyanine and selenium function as equivalent photoconducts. There is no such recognition here that the device of Kanno can function as an implantable pulse generator.

In summary, Lee et al. in view of Kanno fails to teach or suggest the claimed invention. First, neither Lee et al. nor Kanno teach heterologous promoter constructs using an electrically responsive promoter element. Second, based on the operability of Kanno et al., there would be no motivation to make a heterologous promoter system in cells that are implanted with an implantable pulse generator. And third, Kanno and Pahwa upon further scrutiny do not stand the test as being art recognized equivalents. Based upon the submitted arguments, Applicants' respectfully request that the present rejection over Lee et al., and in view of Kanno et al., and in further view of Pahwa et al., be removed.

Rejection under 35 USC 103(a) as being unpatentable over Lee et al., taken with Kanno et al. and Pahwa et al. in view of both McDonough et al. and Allen.

Claims 1-4, 7-25, 39, 40, and 43-48 were rejected under 35 USC 103(a) as being unpatentable over Lee et al., taken with Kanno et al. and Pahwa et al., as applied to claim 1-4, 7-10, 13, 14, 23-25, 39, 40, and 43-48, in view of both McDonough et al . (J. Biol Chem., 1997, 272 : 24046-24053) and Allen (Ann Thorac Surg., 1999, 68 : 1924-1925). The Examiner has indicated that Lee et al., taken with Kanno et al. and Pahwa et al. does not teach the limitation of using an electrically enhancer element selected from the ANF 5' non-coding region. However, at the time the invention was made, McDonough et al. did teach elements derived from the ANF 5' non-coding region driving the expression of the luciferase gene upon electrical stimulation (page 24047, Experimental Procedures). Thus, the Examiner concludes it would have been obvious for one of ordinary skill in the art, at the time the invention was made, to replace the VEGF enhancer with the enhancer of McDonough et al. (i.e., the enhancer element is heterologous to the coding sequence or to the promoter sequence) with a reasonable expectation of success. The Examiner finds that one of ordinary skill in the art would have been motivated to employ such a chimeric enhancer-promoter containing in conjunction with an electrical pulse generator for controlled delivery of genes, and that one skilled in the art would have a reasonable expectation of success in making and using the combined composition because the enhancer element selected from the ANF 5' noncoding

region was proven to promote gene expression as desired, upon electrical stimulation. The Examiner has further indicated that Lee et al., taken with Kanno et al. and Pahwa et al. does not teach the limitation of a tissue specific promoter. However, at the time the invention was made, Allen was cited for teaching organ-selective local delivery of therapeutic genes (Abstract, page 1924 bridging page 1925, column 1, first paragraph). Thus, the Examiner concludes it would have been obvious for one of ordinary skill in the art, at the time the invention was made, to genetically alter cells with a construct comprising the VEGF electrically responsive enhancer and link it to a tissue specific promoter for local delivery, as taught by Allen, with a reasonable expectation of success. Upon that basis, the Examiner concludes that one of ordinary skill in the art would have been motivated to employ such a chimeric enhancer-promoter construct in conjunction with an electrical pulse generator for controlled delivery of genes to specific organs/tissue, since the electrical pulse stimulation of a promoter was crucial for modulation of gene expression as taught by the primary reference. One of ordinary skill in the art would have a reasonable expectation of success in making and use such as the combined composition because electrically responsive enhancers are proven to promote gene expression as desired, upon electrical stimulation. Thus, the Examiner finds the claimed invention was prima facie obvious at the time the invention was made.

As previously reviewed, Lee et al. teaches delivery of a VEGF gene through a retroviral promoter into the ventricular wall of immunodefficient mice. No where does Lee et al. teach that the retroviral promoter is an electrically responsive promoter. However, Kanno et al. was cited by the Examiner for teaching the induction of the VEGF gene in electrical pulse stimulated C2C12 cells as an electrically responsive promoter system. But as previously discussed, Kanno et al. only teaches induction of expression of the endogenous VEGF gene using electrical stimulation in non-genetically engineered cells. It was not shown in Kanno et al. to be effective in engineered cells, nor was it shown to be applicable in heterologous promoter systems. Both these limitations are contained in the claims.

In addition, after reading Kanno et al. there would be no motivation for transfecting the VEGF gene into the tissue to enhance expression of this gene by electrical stimulation, wherein the gene is already present in the tissue and responsive to electrical stimulation. Because the gene is present and shown to be responsive to electrical stimulation, there is no motivation to create a heterologous promoter system. Electrical stimulation of the endogenous gene would have been achieved without creating the required genetically engineered cells and then coupling these to an implantable pulse generator. Neither Lee et al. or Kanno teach heterologous promoter constructs using an electrically responsive promoter element or their implantation or the coupling of this system to an implantable pulse generator. The deficiency of Pahwa et al. and Kanno et al. as being art recognized equivalents was previously discussed.

In view of the deficiencies of Lee et al., Kanno et al., and Pahwa, the Examiner has combined these references with McDonough et al. and Allen. Applicants wish to point out that the standing claims are distinguishable and patentable over Lee et al., taken with Kanno et al. and Pahwa et al., in view of both McDonough et al. and Allen. Although McDonough teaches the use of electrical stimulation, it fails in the same manner as the previously discussed Pahwa reference. McDonough fails to teach or suggest a device that could be used as an implanted pacemaker. In particular, McDonough cites to his earlier Journal of Biochemistry Paper for the device used to stimulate cells (see previously submitted IDS for McDonough et al., J. Biol. Chem. 269(13):9466-9472, 1994). This device shows cultured cells utilizing silver chloride electrodes inserted through holes in the lid of a multiculture dish. The circuits were completed by linking the individual wells with thin strips of 1% agarose gel permeated with culture medium. Although McDonough refers to pacing cells, it clearly is in context of pacing cultured cells. No where in McDonough is there a clear teaching or suggestion of use of an implantable electrical stimulation device or the requirements for such a device, or that there is an art recognized equivalent. Further, McDonough does not teach the use of transplanting these cells or coupling the implanted cells to a pulse generator. The addition of the Allen reference was to suggest a means for tissue regulated expression; however, after carefully reading through this paper, and at the locations indicated by the Examiner, the Applicants do not see such a teaching. No where in Allen could they find a direct teaching of tissue regulated gene expression suggested by the Examiner, and if so, it certainly was not an enabled teaching. Therefore, Allen adds nothing more to McDonough further in view of Lee et al. and Kanno et al. and Pahwa et al. These references singularly and collectively fail to teach or suggest the claimed invention.

The Applicants note that the Examiner relies on 5 references to try to teach each piece of the invention, but fails to show the motivation within the references that would lead one skilled in the art with all motivations to combine all of these references. Further, because Lee et al. in view of Kanno et al., Pahwa et al.,

McDonough et al., and Allen fails to teach or suggest the claimed invention. Applicants' respectfully request the present rejection be removed.

Claim Rejections Under 35 USC § 103 in view of Lee et al. taken with Kanno et al. and Pahwa et al. and Kaye.

Claims 1-4, 7-10, 13, 14, 23-26, 39, 40, and 43-48 were rejected under 35 U.S.C. 103(a) as being unpatentable over Lee et al. taken with Kanno et al. and Pahwa et al., as applied to claims 1-4, 7-10, 13, 14, 23-25, 39, 40, and 43-48 above, in further view of Kaye et al . (Circ Res., 1996, 78:217-224). The Examiner has indicated that Lee et al. taken with Kanno et al. and Pahwa et al. do not teach a coding sequence selected from the group recited in claim 26. However, the Examiner points out that at the time the invention was made, Kaye et al. did teach activation of constitutive nitric oxide synthesis (NOS) in rat myocytes upon electrical stimulation (Abstract, page 219, bridging p. 220, column 1). Thus, the Examiner concludes it would have been obvious for one of ordinary skill in the art, at the time the invention was made, to employ and genetically engineer a cell with a construct comprising the VEGF linked to a DNA encoding for NOS to modulate NOS expression upon electrical stimulation, with a reasonable expectation of success. The Examiner indicates that one of ordinary skill in the art would have been motivated to employ such a chimeric construct in conjunction with an electrical pulse generator for controlled expression of NOS, since Kaye et al. teach that NOS participates in the regulation of contractile function of cardiac muscle via nitric oxide synthesis, which in turn, mediates muscarinic cholinergic signaling in cardiac myocytes and specialized pacemaker tissue, and modifies contractile response to R-adrenergic stimulation (page 217 bridging page 218). One of ordinary skill in the art would have been expected to have a reasonable expectation of success in making and use such as the combined composition because the VEGF promoter is proven to modulate gene expression as desired, upon electrical stimulation. Thus, the claimed invention was prima facie obvious at the time the invention was made.

The arguments against the combination of Lee et al. taken with Kanno et al. and Pahwa et al. have already been presented (see earlier arguments). Briefly, neither Lee et al. nor Kanno et al. teach heterologous promoter constructs using an electrically responsive promoter element. Use of Pahwa was argued does not provide the art recognized equivalents of an implantable pacemaker. Kaye et al. was added because the features of using the nitric oxide synthesis gene (NOS) found in claim 26 was not found in Lee et al. taken with Kanno et al. and Pahwa et al.

Kaye's studies involve *in vitro* electrical stimulation of isolated heart myocytes. These cells were used in their native condition. No genetic engineering of the cells was performed or suggested. Kaye's experiments suggest that the nitric oxide synthesis gene would respond to *in vitro* pacing; however, the reference fails to adequately describe an implantable pacemaker tied to implanted cells that are

genetically engineered to a heterologous system. Thus, Applicants argue that Kaye fails to provide the necessary elements sought by the Examiner.

In view of the amendments to the claims, and the arguments submitted above distinguishing the present claimed invention over Lee et al., in view of Kanno et al., Pahwa et al. and Kaye et al., Applicants' respectfully request the present rejection be removed.

Conclusion

In view of the submitted amendments and discussion of the prior art, Applicants believe all the present rejections have been overcome, and respectfully request that the present application be allowed to issue.

Respectfully submitted for,

Padua, et al.

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PENDING CLAIMS AS AMENDED (MARKED UP VERSION)

What is claimed is:

1. [currently amended] A [therapeutic] delivery system comprising an implanted electrical pulse generator operably coupled with genetically engineered cells that have been transplanted into a mammalian tissue, wherein said genetically engineered cells further comprise a target gene that has been operably coupled in vitro to a heterologous electrically responsive promoter capable of enhancing transcription of said target gene.

2. [currently amended] A [therapeutic] delivery system of claim 1 wherein the electrical pulse generator provides a subthreshold stimulation.

3. [currently amended] A [therapeutic] delivery system of claim 1 wherein the electrical pulse generator provides a threshold stimulation.

4. [currently amended] A [therapeutic] delivery system of claim 1 wherein the electrical pulse generator provides stimulation to the tissue from attached electrodes.

5. [withdrawn] A [therapeutic] delivery system of claim 1 wherein the electrical pulse generator provides stimulation to the tissue without attached electrodes using Eddy currents induced by time varying magnetic fields.

6. [withdrawn] A [therapeutic] delivery system of claim 1 wherein the electrical pulse generator provides stimulation to the tissue without attached electrodes using displacement currents induced by time varying electrical fields applied externally.

7. [currently amended] A [therapeutic] delivery system of claim 1 wherein the electrical pulse generator is a pacemaker.

8. [currently amended] A [therapeutic] delivery system of claim 1 wherein the electrical pulse generator is implanted.

9. [cancelled] A therapeutic delivery system of claim 1 wherein the electrical pulse generator is external

10. [currently amended] A [therapeutic] delivery system of claim 1 wherein the electrical pulse generator is externally controlled.

11. [currently amended] A [therapeutic] delivery system of claim 1 wherein the electrical response promoter contains an electrically responsive enhancer element that is heterologous to the coding sequence.

12. [currently amended] A [therapeutic] delivery system of claim 1 wherein the electrical response promoter contains an electrically responsive enhancer element heterologous to the promoter sequence.

13. [currently amended] A [therapeutic] delivery system of claim 1 wherein the electrically responsive promoter is responsive to subthreshold stimulation.

14. [currently amended] A [therapeutic] delivery system of claim 1 wherein the electrically responsive is responsive to threshold stimulation.

15. [currently amended] A [therapeutic] delivery system of claim 1 wherein the electrically responsive promoter contains an electrically responsive enhancer element selected from the ANF 5' non-coding region.

16. [currently amended] A [therapeutic] delivery system of claim 1 wherein the electrically responsive promoter comprises an ERE operably linked to a tissue specific promoter.

17. [currently amended] A [therapeutic] delivery system of claim 1, wherein said promoter is a cardiac-specific promoter.

18. [currently amended] A[therapeutic] delivery system of claim 17, wherein said promoter is selected from the group consisting of the ANF promoter, alpha-MHC.sub.5.5 promoter, alpha-MHC.sub.87 promoter, and human cardiac actin promoter.

19. [currently amended] A [therapeutic] delivery system of claim 1, wherein said promoter is a kidney specific promoter.

20. [currently amended] A [therapeutic] delivery system of claim 1, wherein said promoter is a brain specific promoter.

21. [currently amended] A [therapeutic] delivery system of claim 1, wherein said promoter is selected from the group consisting of aldolase C promoter, and tyrosine hydroxylase promoter.

22. [currently amended] A [therapeutic] delivery system of claim 1, wherein said promoter is a vascular endothelium specific promoter.

23. [currently amended] A [therapeutic] delivery system of claim 1, wherein said electrical response promoter, or fragment thereof, is selected from the group consisting of ANF, VEGF, acetylcholine receptor, troponin, NOS3, cytochrome c, COX, CPT-1, hsp70, and skm2.

24. [currently amended] A [therapeutic] delivery system of claim 1 wherein the genetically engineered cells are mammalian cells.

25. [currently amended] A [therapeutic] delivery system of claim 1 wherein the genetically engineered cells are selected from the group of C2C12.

26. [currently amended] A [therapeutic] delivery system of claim 1 wherein said coding sequence is selected from the group consisting of tissue plasminogen activator (tPA), nitric oxide synthase (NOS), Bcl-2, superoxide dismutase (SOD), and catalase.

27. (withdrawn) An expression vector, comprising an electrical response enhancer element, a tissue specific promoter heterologous to the element, and a coding sequence, wherein said promoter is operably linked to said coding sequence and said element is effective to cause expression of said coding sequence.

28. (withdrawn) An expression vector of claim 27, wherein said expression vector is a plasmid.

29. (withdrawn) An expression vector of claim 27, wherein said expression vector is an adenovirus vector.

30. (withdrawn) An expression vector of claim 27, wherein said expression vector is a retrovirus vector.

31. (withdrawn) An expression vector of claim 27, wherein said coding sequence is a viral thymidine kinase coding sequence.

32. (withdrawn) An expression vector of claim 31, wherein said viral thymidine kinase coding sequence encodes herpes simplex viral thymidine kinase.

33. (withdrawn) An expression vector of claim 27, wherein said coding sequence encodes luciferase.

34. (withdrawn) An apparatus for testing cells comprising an upper plate electrode, a lower plate electrode, and a porous membrane which is positioned between said upper and lower plate electrodes during operation.

35. (withdrawn) An apparatus of claim 34 wherein the upper plate electrode is the same size as the lower plate electrode.

36. (withdrawn) An apparatus of claim 34 wherein the lower plate electrode forms a receiving means for the porous membrane.

37. (withdrawn) An apparatus of claim 34 wherein the porous membrane supports cells between said upper and lower plate electrodes.

38. (withdrawn) An apparatus of claim 34 which is operably coupled to a pulse generator.

39. [currently amended] A method [of treating a patient] of delivering genetically engineered cells to a tissue comprising providing [the patient with a]at the tissue site an implantable electrical pulse generator operably coupled with genetically engineered cells that have been transplanted into a patient tissue, wherein said genetically engineered cells further comprise a target gene that has been operably coupled in vitro to a heterologous electrically responsive promoter capable of enhancing transcription of said target gene.

40. [currently amended] A method providing [a patient with] an implantable electrical pulse generator operably coupled with genetically engineered cells that have been transplanted into [a patient] the tissue of a host, wherein said genetically engineered cells further comprise a target gene operably coupled in vitro to a heterologous electrically responsive promoter capable of enhancing transcription of said target gene.

41. [canceled] A genetically engineered cell of claims 1, 39 or 40 wherein genetically engineered cells a transplanted into the patient tissue.

42. [canceled] A method of either claims 1, 39 or 40 wherein genetically engineered cells are obtained by transfecting the cells of the patient tissue.

43. [currently amended] A [therapeutic] delivery system of Claim 1 [method of either claims 1, 39 or 40] wherein the transfected tissues are independently selected from, epithelial tissue, endothelial tissue, or mesodermal tissue.

44. [currently amended] A [therapeutic] delivery system of Claim 1 wherein the genetically engineered cells are independently selected from the group consisting skeletal muscle cells, heart muscle cells, smooth muscle cells, pluripotent stem cells, mesodermal stem cells, myoblast, fibroblasts, cardiomyoctes, cholinergic neurons, adrenergic neurons, and peptidergic neurons, glial cells, astrocytes, oligodendrocytes, schwann cells, vascular endothelial cells, synovial cells, acinar cells, hepatocytes, chondrocytes, osteoblasts, osteoprogenitor cells, nucleus pulposus cells, and cells of the intervertebral disk.

45. [currently amended] A method of [treating a patient] delivering genetically engineered cells to a tissue of Claim 39 wherein the transfected tissues are independently selected from, epithelial tissue, endothelial tissue, or mesodermal tissue.

46. [currently amended] A method of [treating a patient of] delivering genetically engineered cells to a tissue Claim 39, wherein said genetically engineered cells are independently selected from the group consisting skeletal muscle cells, heart muscle cells, smooth muscle cells, pluripotent stem cells, mesodermal stem cells, myoblast, fibroblasts, cardiomyoctes, cholinergic neurons, adrenergic neurons, and peptidergic neurons, glial cells, astrocytes, oligodendrocytes, schwann cells, vascualr endothelial cells, snopvial cells, acinar cells, hepatocytes, chondrocytes, osteoblasts, osteoprogenitor cells, nucleus pulposus cells, and cells of the intervertebral disk.

47. [currently amended] A method of delivering genetically engineered cells to a tissue of a host [treating a patient] of Claim 40 wherein the transfected tissues are independently selected from, epithelial tissue, endothelial tissue, or mesodermal tissue.

48. [currently amended] A method providing [a patient with] an electrical pulse generator operably coupled with genetically engineered cells that have been transplanted into the tissue of a host [in a patient tissue] of Claim 40, wherein the genetically engineered cells are independently selected from the group consisting skeletal muscle cells, heart muscle cells, smooth muscle cells, pluripotent stem cells, mesodermal stem cells, myoblast, fibroblasts, cardiomyoctes, cholinergic neurons, adrenergic neurons, and peptidergic neurons, glial cells, astrocytes, oligodendroytes, schwann cells, vascular endothelial cells, synovial cells, acinar cells, hepatocytes, chondrocytes, osteoblasts, osteoprogenitor cells, nucleous pulposus cells, and cells of the intervertebral disk.